process in which:

$$Y_\infty - Y_i = \sum_{j=1}^{n} A_j e^{-k_j t_i} \qquad \text{(Eq. A1)}$$

Y_∞ can be found, by applying Prony's method, as:

$$Y_\infty = \frac{\begin{vmatrix} Y_i & Y_i - Y_{i+1} & \cdots & Y_{i+n-1} - Y_{i+n} \\ \vdots & \vdots & & \vdots \\ Y_{i+n} & Y_{i+n} - Y_{i+n+1} & \cdots & Y_{i+2n-1} - Y_{i+2n} \end{vmatrix}}{\begin{vmatrix} 1 & Y_i - Y_{i+1} & \cdots & Y_{i+n-1} - Y_{i+n} \\ \vdots & \vdots & & \vdots \\ 1 & Y_{i+n} - Y_{i+n+1} & \cdots & Y_{i+2n-1} - Y_{i+2n} \end{vmatrix}} \qquad \text{(Eq. A2)}$$

for any value of i. Hildebrand (3) noted that Prony's method is sensitive to errors in the Y_i values. This fact and the fact that the error of the estimate of Y_∞ decreases as the time interval between samples increases can be rationalized easily by considering any nonunit column of the determinants that appear in the numerator and denominator of Eq. A2.

Since the sequence $\{Y_i\}$ is approaching a finite limit, Y_∞, as $i \to \infty$, Cauchy's theorem tells us that $(Y_i - Y_{i+1}) \to 0$ as $i \to \infty$. As a result, each column of the determinants in Eq. A2 approaches $(0 \cdots 0)^T$ and the value of Y_∞ given by Eq. A2 becomes indeterminate. Before reaching this limiting situation, the error in the estimate of Y_∞ grows larger and larger as the difference between Y_i and Y_{i+1} grows smaller and smaller. Clearly, $Y_i - Y_{i+1}$ will be larger when $\Delta t = t_{i+1} - t_i$ is larger.

If x is defined as:

$$x = e^{-k\,\Delta t} \qquad \text{(Eq. A3)}$$

then it is not difficult to show that:

$$Y_i - Y_{i+1} = Y_\infty x^i (x - 1) \qquad \text{(Eq. A4)}$$

Since $x < 1$, Eq. A4 shows that the exact difference, $Y_i - Y_{i+1}$, is a small number for most i values. If ϵ_i and ϵ_{i+1} are the absolute errors associated with measurements of Y_i and Y_{i+1}, then the experimental value of the difference shown in Eq. A4 possibly could be:

$$(Y_i - Y_{i+1})^{exp} = |\epsilon_i| + |\epsilon_{i+1}| + Y_\infty x^i (x - 1) \qquad \text{(Eq. A5)}$$

That is, the elements of the determinants in Eq. A2 can easily be dominated by the error contained in the measured Y_i values, thus leading to serious errors in the estimates of Y_∞.

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Synthesis and Antitumor Activity of 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyrates

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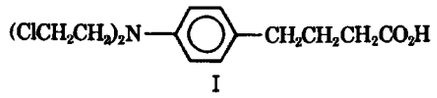
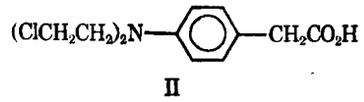
Abstract □ Ten 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyrates were synthesized and evaluated for antitumor activity. The 2-phenoxyethyl ester exhibited activity against P-388 lymphocytic leukemia, and the *n*-butyl and *n*-pentyl esters exhibited activity against L-1210 lymphoid leukemia in initial screening tests.

Keyphrases □ Chlorambucil analogs—synthesis and evaluation for antitumor activity □ Structure–activity relationships—chlorambucil analogs, synthesis and testing for antitumor activity □ Antitumor activity—chlorambucil analogs, synthesis and evaluation for activity □ 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyrates—synthesis and evaluation for antitumor activity

Chlorambucil [4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyric acid, I] exhibits marked antitumor activity, primarily by virtue of its properties as an alkylating agent. It has been used extensively in the treatment of chronic



I



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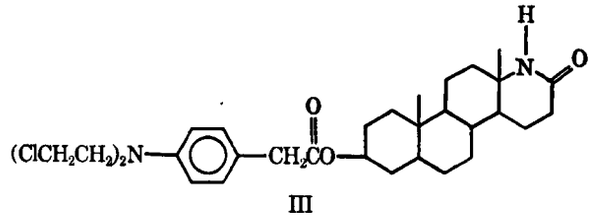

III

lymphocytic leukemia (1, 2) and ovarian carcinoma (3, 4) as well as in combination with other drugs (5–7).

BACKGROUND

Recent reports suggested that various derivatives of cytotoxic aralkyl acids, notably esters and amides, have significantly decreased toxicity and enhanced therapeutic indexes compared to the parent acids. This observation also was noted[1] for some of the compounds reported in this paper. Niculescu-Duvaz *et al.* (8) studied nitrogen mustards of various methylbenzoic acids and noted that the methyl ester of one isomer (unavailable as the free acid) exhibited an LD_{50} value of 300 mg/kg compared to a range of 15–62 mg/kg for the other acids, with antineoplastic activity similar in degree to that of the other compounds.

Reports have appeared on phenesterine, estradiol mustard, and

[1] J. H. Billman to W. F. Dunning, Papanicolaou Cancer Research Institute, Miami, Fla., personal communication, January 1975.

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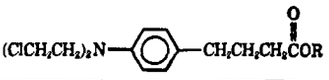
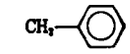
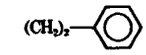
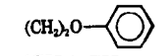
Table I—4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyrates



Compound	R	Formula	Yield (Pure), %	Melting Point	Analysis, %			IR, Carbonyl Stretching Band, cm^{-1}
						Calc.	Found	
IV	$(CH_2)_7CH_3$	$C_{22}H_{35}Cl_2NO_2$	95.3	Oil[a]	N	3.36	3.17	1730
V	$(CH_2)_5CH_3$	$C_{20}H_{31}Cl_2NO_2$	58.6	Oil[a]	N	3.59	3.44	1725
VI	$CH_2CH(CH_3)_2$	$C_{18}H_{27}Cl_2NO_2$	50.4	Oil[a]	N	3.88	3.71	1725
VII		$C_{21}H_{25}Cl_2NO_2$	81.5	39.5–40.0°	N	3.55	3.42	1740
VIII		$C_{22}H_{27}Cl_2NO_2$	81.4	Oil[a]	N	3.42	3.19	1720
IX		$C_{22}H_{27}Cl_2NO_3$	74.4	Oil[a]	N	3.30	3.25	1745
X	$(CH_2)_3CH_3$	$C_{18}H_{27}Cl_2NO_2$	79.6	Oil[a]	N	3.88	3.87	1740
XI	$CH(CH_2)_5CH_3$ CH_3	$C_{22}H_{35}Cl_2NO_2$	67.1	Oil[a]	N	3.36	3.25	1735
XII	$(CH_2)_4CH_3$	$C_{19}H_{29}Cl_2NO_2$	50.0	Oil[a]	N	3.73	3.59	1725
XIII	$CH(CH_3)_2$	$C_{17}H_{25}Cl_2NO_2$	46.5	Oil[a]	N	4.04	3.89	1730

[a] The oils decompose upon heating, even *in vacuo*, so more accurate data are not available.

dehydroepiandrosterone mustard, which are esters of 4-[*p*-[bis(2-chloroethyl)amino]phenyl]acetic acid (II) (9–13); these compounds appear to be significantly less toxic and more suitable for use than II. Wampler and Catsoulacos (14) reported that the homo aza-steroidal ester of *p*-[bis(2-chloroethyl)amino]phenylacetic acid (III) was active against L-1210 and P-388 leukemias, while the parent compound, phenestrin, was relatively inactive.

In a comparison of nitrogen mustard derivatives of aryl amides, phenols, and aryl acids, the amide and ester derivatives generally appeared to be significantly less toxic and more selective (15, 16). The possibility was suggested that the active component is the original acid, formed by enzymatic hydrolysis of the appropriate ester or amide linkage. Numerous studies have been conducted on the premise that a lipophilic compound can be transported across cell walls considerably faster than corresponding ionic forms, apparently by the involvement of an appropriate carrier (17–21). Repta *et al.* (22) observed that an antitumor agent with optimal activity against a tumor implanted in a location removed from the intraperitoneal drug administration site must be more lipophilic than one that is optimum for an intraperitoneally implanted tumor.

Quantitative structure–activity regression analysis of various nitrogen mustards showed a correlation between the hydrolysis rate of the compound and its toxicity and activity against L-1210 and P-388 leukemias, B-16 melanoma, and WA-256 tumors (23–25). Higher lipophilic requirements were found for the WA-256 system, which suggests that solid tumors may require more lipophilic drugs.

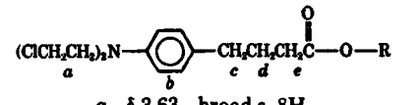
Billman and Roehrig (26) prepared several compounds that were structurally similar to chlorambucil but with the carboxylic acid group converted to various amide groups. Several of the compounds exhibited antitumor activity during initial stages of testing. The present report describes the synthesis of several similar compounds related to chlorambucil where the carboxylic acid group was converted into various ester groups. The ester groups were chosen to include linear, branched primary, secondary, and primary aralkyl esters. One aralkyl ether was included because of previous interest in the alcohol as a potential antiviral agent.

EXPERIMENTAL[2]

The esters were prepared from chlorambucil and the appropriate alcohol by reaction with *p*-toluenesulfonic acid in benzene or toluene under azeotropic conditions to remove water formed during the reaction. Formation of the esters was confirmed by the appropriate IR spectral bands, NMR data, and elemental analysis. The analytical data for the compounds are given in Tables I and II.

For the preparation of 2-phenoxyethyl-4-[*p*-[bis(2-chloroethyl)-amino]phenyl]butyrate (IX), chlorambucil (8.00 g, 0.263 mole) and 2-phenoxyethanol (3.63 g, 0.263 mole) were dissolved in 100 ml of dry toluene. *p*-Toluenesulfonic acid (1.0 g, 0.0058 mole) was added, and the mixture was refluxed with stirring for 5.5 hr in an apparatus equipped with a Dean-Stark trap for water removal (27). The solvent was evaporated *in vacuo*, and the resulting mixture was taken up in a minimum amount of petroleum ether (30–60°) and chromatographed over neutral alumina. The solvent was removed from the fraction containing the ester *in vacuo*, resulting in the ester. In several cases, the resulting oil was rechromatographed to obtain material of satisfactory purity.

Table II—NMR Data for 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]-butyrate



a δ 3.63 broad s, 8H
b δ 6.75 q, 4H
c δ 2.15 t, 2H
d δ 1.87 m, 2H
e δ 2.43 t, 2H

Compound	R-Group Data
IV	δ 4.09, t, 2H δ 0.89, t, 3H δ 1.5, m, 12H
V	δ 4.22, t, 2H δ 1.10, t, 3H δ 1.5, m, 8H
VI	δ 3.83, d, 2H δ 0.95, d, 6H δ 2, m[a], 1H
VII	δ 7.29, s, 5H δ 5.05, s, 2H
VIII	δ 7.17, s, 5H δ 4.20, t, 2H δ 2.80, t, 2H
IX	δ 7.10, m, 5H δ 4.23, m, 4H
X	δ 0.91, t, 3H δ 4.03, t, 2H δ 1.5, m, 4H
XI	δ 4.7, m, 1H δ 1.20, d, 3H δ 0.91, t, 3H δ 1.5, m, 10H
XII	δ 4.02, t, 2H δ 0.90, t, 3H δ 1.5, m, 6H
XIII	δ 5.00, m, 1H δ 1.24, d, 6H

[a] Buried under c protons.

[2] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are corrected. IR spectra were obtained on a Perkin-Elmer 237B or Beckman Acculab 6 spectrophotometer. NMR spectra were obtained on a Varian A-60A, Perkin-Elmer R-24, or *JEOLCO* 100-MHz instrument. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Table III—Antitumor Data of 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyrates[a]

Compound	NSC Number	L-1210		P-388		IRC 741		FAA 101		R-3398	
		mg/kg	T/C, %	mg/kg	T/C, %	mg/kg	T/C, %	mg/kg	T/C, %	mg/kg	T/C, %
IV	208419	400	111			1.5	118	2.0	—	2.0	21
		200	108								
		100	104								
V	208420	400	124								
		200	111								
		100	114								
VI	208421	200	111								
		100	121								
VII	209852	400	121								
		200	108								
		100	132								
VIII	209853	400	112								
		200	112, 122								
		100	132, 124								
		50	114								
		25	111								
IX	236259			50	200, 174						
				25	223						
				12.5	155						
X	236260	100	130								
		50	144, 125								
		25	135, 135								
XI	236261	400	100								
		200	158, 106, 104								
		100	135, 116, 104								
		50	122, 104								
XII	236262	100	88, 86								
		50	135, 123, 127								
		25	139, 128, 131								
		12.5	118								
XIII						1.5	123	2.0	4	2.0	51

[a] L-1210 and P-388 tumors were screened by the National Cancer Institute. IRC 741, FAA 101, and R-3398 tumors were screened by Dr. W. F. Dunning, Papanicolaou Cancer Research Institute, Miami, FL 33123.

RESULTS AND DISCUSSION

Antitumor data for each compound are listed in Table III. Several compounds showed slight activity against L-1210 lymphoid leukemia. Compound IX showed some activity against P-388 lymphocytic leukemia. Compounds IV and XII also exhibited some activity against IRC 741 (acute leukemia intraperitoneal) but were essentially inactive against FAA 101 (stomach sarcoma intraperitoneal) and R-3398 (reticular cell sarcoma intraperitoneal), which are solid tumors.

On the basis of the antitumor results obtained to date, some tentative conclusions may be reached. The short-chain linear esters possess greater activity against L-1210 leukemia than do the longer chain homologs. Branched primary and secondary esters and simple aralkyl esters show no significant activity against L-1210 leukemia. However, the one aralkoxy ester shows significant activity against P-388 leukemia; its activity against L-1210 leukemia is unknown. Further work is indicated with the short-chain linear esters and derivatives of the aralkoxy ester.

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